protein/ligand complexes are essential in exploring this issue; structural determinations of the complexes of interest, however, are often challenging and docking procedure can be carefully designed as an adequate substitute. Prostaglandin endoperoxide H synthases (PGHSs), also known as cyclooxygenases, have been investigated recently in an attempt to understand their half-of-sites enzyme activity and consequent biological significance. PGHSs are heme-containing proteins exist in solution as sequence homodimer, but once their catalytic cycle of cyclooxygenase/peroxidase is initiated, they behave functionally as a heterodimeric manner. Biochemical approach to solve this puzzle has been intensively attempted and suggested their interchangeability of structurally distinct two monomers. This paper presents an analysis of PGHS-ligand interactions upon binding either experimentally or virtually and predicts its biological implications.

1338-Pos Board B68

A Novel Aromatic Carboxylic Acid Inhibits Luciferase Enzymatic Activity in Mammalian Cells by Acylation of an Active Regulatory Lysine Residue

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Firefly luciferase (Luc) is widely used as a reporter enzyme in cell-based assays for gene expression. A novel aromatic carboxylic acid, F-53 substantially inhibited the enzymatic activity of Luc in a Luc reporter screening. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and tandem mass spectrometry (MS/MS) analyses showed that F-53 modified Luc at lysine-529 via amidation of the F-53 carboxyl group. The lysine-529 residue of Luc, which plays a regulatory catalytic role, can be acetylated. Luc also has a long-chain fatty acyl-CoA synthase activity. An in vitro assay that involved both recombinant Luc and mouse liver microsomes identified F-53-CoA as the reactive form produced from F-53. However, whereas the inhibitory effect of F-53 is observed in Hela cells that transiently expressed Luc, it is not observed in an in vitro assay that involves recombinant Luc alone. Therefore, insights into the activities of certain mammalian transferases can be translated to better understand the acylation by F-53.

The purification of the interacting proteins with F-53 in mammalian cells was carried out using F-53-immobilized Magnetic FG beads as bait and those were analyzed by using nano-Liquid chromatography coupled with electrospray ionization-quadrupole time-of-flight hybrid mass spectrometry (nano-LC/ESI-QTOF-MS).

1339-Pos Board B69

Structure Analysis of Histidine Decarboxylase in Complex with Inhibitors

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Histamine is a bioactive amine responsible for a variety of physiological reactions, including allergy, gastric acid secretion, and neurotransmission. In mammals, histamine production from histidine is catalyzed by histidine decarboxylase (HDC). Mammalian HDC is a pyridoxal 5'-phosphate (PLP)-dependent decarboxylase and belongs to the same family as mammalian glutamate decarboxylase (GAD) and mammalian aromatic L-amino acid decarboxylase (AroDC). The decarboxylases of this family function as homodimers and catalyze the formation of physiologically important amines like γ-aminobutyric acid (GABA), dopamine, and serotonin via decarboxylation of glutamate, 3,4-dihydroxyphenylalanine (DOPA), and 5-hydroxytryptophan, respectively. Despite high sequence homology, both AroDC and HDC react with different substrates. For example, AroDC catalyzes the decarboxylation of several aromatic L-amino acids, but has little activity on histidine. Although such differences are known, the substrate specificity of HDC has not been extensively studied because of the low levels of HDC in the body and the instability of recombinant HDC, even in a well-purified form. However, knowledge about the substrate specificity and decarboxylation mechanism of HDC is valuable from the viewpoint of drug development, as it could help lead to designing of novel drugs to prevent histamine biosynthesis.

The structure of the mammalian HDC has not yet been reported. The three-dimensional structure will provide invaluable insights into the molecular basis of the histidine substrate specificity. We have determined the crystal structure of human HDC in complex with an inhibitor, histidine methyl ester (HME). This structure showed the detailed features of the PLP-inhibitor adduct (external aldimine) in the active site of HDC. Moreover, a comparison of the structure of HDC with that of AroDC revealed a key residue, Ser354, for substrate specificity. These data provided insight into the molecular basis for substrate recognition among the PLP-dependent L-amino acid decarboxylases.

1340-Pos Board B70

Covalent Docking of Large Libraries for the Discovery of Chemical Probes

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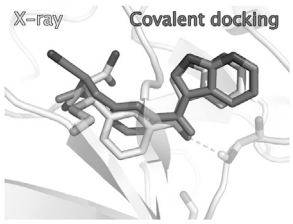
Chemical probes that form a covalent bond with a protein target often show enhanced selectivity, potency, and utility for biological studies. Despite these advantages, protein-reactive compounds are usually avoided in high-throughput screening campaigns. Here we describe a general method (DOCKovalent) for screening large virtual libraries of electrophilic small molecules. We apply this method prospectively to discover reversible covalent fragments that target distinct protein nucleophiles: (1) the catalytic serine of AmpC β-lactamase, screening 23,000 boronic acids, and (2) a noncatalytic cysteine in RSK2 and MSK1 kinases, screening 12,000 cyanoacrylamide fragments. In both virtual screens, we identify submicromolar hits with high ligand efficiency and cellular activity. Crystal structures of several selected inhibitors bound to AmpC and RSK2 confirm the docking predictions. As covalent virtual screening may have broad utility for the rapid discovery of chemical probes, we have made the method freely available through an automated web server.



Membrane Structure, Folding, and Design

1341-Pos Board B71

Thermodynamic and Functional Analysis of ClC Dimerization

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A wide variety of integral membrane proteins form oligomeric structures within the membrane. For proteins where each monomer makes up an independent active unit, the mechanistic reasons for oligomerization are not always clear. We are attempting to monomerize the normally dimeric members of a fluoride-specific ClC clade through the use of directed mutagenesis, and determine whether dimerization is required for folding and/or function. These partially monomerized constructs are then being used as a tool to analyze the energetics of association and folding in the membrane. Very little is known about the energetics of folding for large, multispanning integral membrane proteins due to the experimental difficulties of probing such systems. Examining the energetics of dimerization of well-folded monomers may allow us to circumvent many of these difficulties. The energetics of intra-membrane protein-protein interaction that drive the folding of individual monomers will also determine the energetics of dimerization. Dimerization can be analyzed, however, without needing to either grossly denature the protein or overcome hysteresis and proper membrane reinsertion upon refolding. This system of reversible oligomerization will allow us to probe many previously unmeasureable aspects of membrane protein folding, and act as a platform from which to examine the effects of mutation and membrane composition.

1342-Pos Board B72

Polar Interactions Trump Hydrophobicity in Stabilizing a Membrane-Interacting Protein

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The vast majority of membrane-bound proteins consist of bundles of hydrophobic α-helical transmembrane domains. How these proteins adopt their native, biologically active structures in the apolar milieu of a membrane is increasingly well understood. However, it is still under debate to what extent polar interactions contribute to membrane-protein stability. For example, the membrane-interacting protein Mistic exhibits a net charge of −12 at pH 7 but, in NMR experiments, displays a concentric ring of interactions with apolar detergent tails typical of more hydrophobic membrane proteins. Moreover, as a fusion tag, Mistic supports the production and membrane insertion of integral membrane proteins in *Escherichia coli*. Thus, in spite of its unusually polar surface, Mistic in many aspects behaves like a conventional membrane protein.

Here, we demonstrate that, upon addition of urea, Mistic unfolds reversibly form detergent micelles following a two-state equilibrium and exhibits the same unfolded reference state irrespective of the detergent used. Unfolding titrations from alkyl maltoside detergents revealed that alkyl tails of 12 carbon atoms preserve the conformational stability of the protein best. However, on top of such hydrophobic interactions, we discovered that zwitter-ionic and charged